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(54) Title: COMBINATION THERAPY FOR THE TREATMENT OF DIABETES AND OBESITY

(57) Abstract

The combination of the β_3 adrenergic receptor agonist Compound A and a compound which modifies feeding behavior (e.g., the OB protein) is useful in the treatment of obesity and diabetes, either as compounds, pharmaceutically acceptable salts, pharmaceutical compoisition ingredients. Methods of treating obesity and diabetes are also described.

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TITLE OF THE INVENTION

COMBINATION THERAPY FOR THE TREATMENT OF DIABETES AND OBESITY

FIELD OF THE INVENTION

The present invention provides a combination useful in the treatment of obesity and diabetes, either as compounds, pharmaceutically acceptable salts or pharmaceutical composition ingredients. Methods of treating obesity and diabetes are also disclosed. More particularly, the combination of the present invention comprises a β_3 agonist and a compound which modifies feeding behavior (e.g., Ob protein, also known as leptin).

BACKGROUND OF THE INVENTION

Obesity, which can be defined as a body weight more than 20% above the ideal body weight, is a major health concern in Western societies, since it is accompanied by numerous complications such as hypertension, non-insulin dependent diabetes mellitus and arteriosclerosis, which in turn cause heart disease, stroke and premature death. Obesity is the result of a positive energy balance, as a consequence of increased ratio of caloric intake to energy expenditure. The molecular factors regulating food intake and body weight balance are incompletely understood. [B. Staels et al., *J. Biol. Chem.* **270**(27), 15958 (1995); F. Lonnquist et al., *Nature Medicine* **1**(9), 950 (1995)]. Although the genetic and/or environmental factors leading to obesity are poorly understood, several genetic factors have recently been identified.

β-Adrenoceptors have been subclassified as β_1 and β_2 since 1967. Increased heart rate is the primary consequence of β_1-receptor stimulation, while bronchodilation and smooth muscle relaxation typically result from β_2 stimulation. Adipocyte lipolysis was initially thought to be solely a β_1-mediated process. However, more recent results indicate that the receptor-mediating lipolysis is atypical in nature. These atypical receptors, later called β_3-adrenoceptors, are found on the cell surface of both white and brown adipocytes where

their stimulation promotes both lipolysis (breakdown of fat) and energy expenditure.

Early developments in this area produced compounds with greater agonist activity for the stimulation of lipolysis (β_3 activity) than for stimulation of atrial rate (β_1) and tracheal relaxation (β_2). These early developments disclosed in Ainsworth et al., U.S. Patents 4,478,849 and 4,396,627, were derivatives of phenylethanolamines.

Such selectivity for β_3-adrenoceptors could make compounds of this type potentially useful as antiobesity agents. In addition, these compounds have been reported to show antihyperglycemic effects in animal models of non-insulin-dependent diabetes mellitus.

A major drawback in treatment of chronic diseases with β_3 agonists is the potential for stimulation of other β-receptors and subsequent side effects. The most likely of these include muscle tremor (β_2) and increased heart rate (β_1). Although these phenylethanolamine derivatives do possess some β_3 selectivity, side effects of this type have been observed in human volunteers. It is reasonable to expect that these side effects resulted from partial β_1 and/or β_2 agonism.

More recent developments in this area are disclosed in Ainsworth et al., U.S. Patent 5,153,210, Caulkett et al., U.S. Patent 4,999,377, Alig et al., U.S. Patent 5,017,619, Lecount et al., European Patent 427480 and Bloom et al., European Patent 455006.

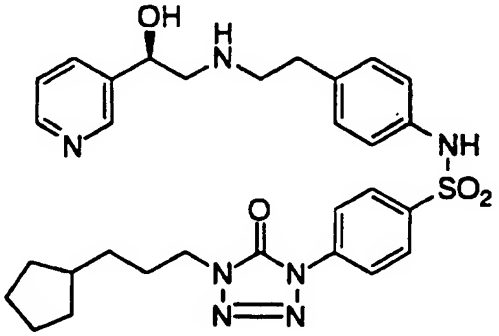
Even though these more recent developments purport to describe compounds with greater β_3 selectivity over the β_1 and β_2 activities, this selectivity was determined using rodents, in particular, rats as the test animal. Because even the most highly selective compounds, as determined by these assays, still show signs of side effects due to residual β_1 and β_2 agonist activity when the compounds are tested in humans, it has become apparent that the rodent is not a good model for predicting human β_3 selectivity.

Recently, assays have been developed which more accurately predict the effects that can be expected in humans. These assays utilize cloned human β_3 receptors which have been expressed in

Chinese hamster ovary cells. See Emorine et al, Science, 1989, 245:1118-1121; and Liggett, Mol. Pharmacol., 1992, 42:634-637. The agonist and antagonist effects of the various compounds on the cultivated cells provide an indication of the antiobesity and antidiabetic effects of the compounds in humans.

These developments have recently led to the discovery of potent and selective β3 agonists useful for treating obesity and diabetes. For example, U.S. Patent No. 5,451,677, issued September 19, 1995, hereby incorporated by reference, describes substituted phenyl sulfonamides which are selective β3 agonists useful for treating obesity and diabetes. These phenyl sulfonamide compounds have been found to be useful in the composition and methods of the instant invention.

More recently, a potent and selective β3 agonist, (R)-N-[4-[2-[[2-Hydroxy-2-(pyridin-3-yl)ethyl]amino]ethyl]-phenyl]-4-[4-(3-cyclopentylpropyl)-5-tetrazolon-1-yl]benzenesulfonamide, hereinafter referred to as Compound A, has been identified.



Compound A

The synthesis of Compound A and its utility for treating obesity and diabetes is described in more detail below, and in PCT International application publication number WO 95/29159, published November 2, 1995, and in U.S. Patent No. 5,561,142, issued October 1, 1996.

In addition to β3 agonists which act on obesity and diabetes by increasing metabolic rate, researchers have recently cloned the mouse OB gene and its human homologue. [Y. Zhang et al., *Nature* 372, 425 (1994)] The OB gene product, i.e., the OB protein (also

known as leptin), a 167 amino acid polypeptide, has been shown to result in a dose- and time-dependent weight loss when administered to mice via intraperitoneal (IP) injection. [M.A. Pelleymounter et al., *Science* **269**, 540 (1995)]. This weight loss effect is attributable to both a reduction in food intake and an increase in energy expenditure. Moreover, since both the mouse and human OB protein have this same effect when administered to mice, the possibility exists that similar effects would also occur in humans. [J.L. Halaas et al., *Science* **269**, 543 (1995)].

It has now been found that a combination of a β3 selective agonist compound and a compound which modifies feeding behavior, for example, by reducing total food intake or by reducing caloric intake or selectively reducing intake of specific components of the diet such as carbohydrates or fats, provides effective therapy for treating obesity and diabetes. More specifically, a combination of Compound A and the OB protein or a derivative thereof is particularly preferred for the treatment of obesity and diabetes.

SUMMARY OF THE INVENTION

The present invention provides a composition comprising a selective β3 agonist and a compound which modifies feeding behavior (e.g., reduces food intake); and the pharmaceutically acceptable salts and esters thereof.

In one embodiment of the invention is the composition comprising a selective β3 agonist and Ob protein or a derivative of the Ob protein; and the pharmaceutically acceptable salts and esters thereof. Preferably, the human Ob protein, or a derivative thereof, is used in combination with a selective β3 agonist.

In a class of the invention is the composition wherein the selective β3 agonist is Compound A, i.e., (R)-N-[4-[2-[[2-hydroxy-2-(pyridin-3-yl)ethyl]amino]ethyl]phenyl]-4-[4-(3-cyclopentylpropyl)-5-tetrazolon-1-yl]benzenesulfonamide; and the pharmaceutically acceptable salts and esters thereof. Preferably, the selective β3 agonist is the dihydrochloride salt of Compound A, i.e., (R)-N-[4-[2-[[2-

hydroxy-2-(pyridin-3-yl)ethyl]amino]-ethyl]phenyl]-4-[4-(3-cyclopentylpropyl)-5-tetrazolon-1-yl]benzenesulfonamide dihydrochloride.

Illustrative of the invention is a method of treating obesity in a subject in need thereof which comprises administering to the subject a therapeutically effective amount of any of the compositions described above.

Exemplifying the invention is a method of treating diabetes in a subject in need thereof which comprises administering to the subject a therapeutically effective amount of any of the compositions described above.

An illustration of the invention is a pharmaceutical composition comprising a therapeutically effective amount of any of the compositions described above and a pharmaceutically acceptable carrier. Further illustrating the invention is the pharmaceutical composition made by combining a selective β3 agonist, a compound which modifies feeding behavior and a pharmaceutically acceptable carrier. Further exemplifying the invention is a process for making a pharmaceutical composition which comprises combining a selective β3 agonist, a compound which modifies feeding behavior and a pharmaceutically acceptable carrier.

An example of the invention is the use of a selective β3 agonist and Ob protein, or a derivative thereof, in the preparation of a medicament for the treatment of obesity.

Another example of the invention is the use of a selective β3 agonist and Ob protein, or a derivative of the Ob protein, in the preparation of a medicament for the treatment of diabetes.

Further illustrating the invention is a drug which is useful for treating obesity, the effective ingredients of the said drug being a selective β3 agonist and Ob protein, or a derivative of the Ob protein.

Further exemplifying the invention is a drug which is useful for treating diabetes, the effective ingredients of the said drug being a selective β3 agonist and Ob protein, or a derivative of Ob protein.

DETAILED DESCRIPTION OF THE INVENTION

This invention is concerned with the combination of certain compounds, or pharmaceutically acceptable salts thereof, for the treatment of obesity and diabetes. Obesity and diabetes mellitus are often treated by encouraging patients to lose weight by reducing their food intake and by increasing their metabolic rate. The ob protein reduces food intake. A β3 selective agonist is targeted to fat and causes increases in metabolic rate. Thus, it has now been found that combination treatment with Ob protein, or a compound that causes increased expression of ob protein, with a β3 selective agonist is advantageous over treatment with either a β3 selective agonist or Ob protein alone in the treatment of obesity and diabetes. Moreover, in addition to its effects on reducing food intake, the ob protein has broad affects. For example, ob protein increases metabolic rate by unknown pathways and lowers glucose and insulin in diabetic mice. As discussed above, β3 selective agonists also increase metabolic rate by specifically targeting fat cells. Thus, when β3 selective agonists are used in combination with the ob gene product, additional beneficial affects on metabolism occur in very obese people.

As used herein, the terms "selective β3 agonist" and "β3 selective agonists" are synonymous and refer to agonists which are selective for the β3 adrenergic receptor subtype over the β1 and β2 adrenergic receptor subtypes in humans. Examples of selective β3 adrenergic agonists are Compound A and the compounds described in U.S. Patent No. 5,541,677. Compound A and additional selective β3 adrenergic agonists which are useful in the compositions and methods of the present invention are described in U.S. Patent No. 5,561,142, issued October 1, 1996 and PCT International patent application Publication No. WO 95/29159, published November 2, 1995.

The term "subject," as used herein refers to an animal, preferably a mammal, most preferably a human, who has been the object of treatment, observation or experiment.

The term "therapeutically effective amount" as used herein means that amount of active compound(s) or pharmaceutical agent(s) that elicits the biological or medicinal response in a tissue, system, animal or human that is being sought by a researcher, veterinarian, medical doctor or other clinician, which includes alleviation of the symptoms of the disease being treated.

The term "diabetes," as used herein, includes both insulin-dependent diabetes mellitus (i.e., IDDM, also known as type I diabetes) and non-insulin-dependent diabetes mellitus (i.e., NIDDM, also known as Type II diabetes).

As used herein, the term "composition" is intended to encompass a product comprising the specified ingredients in the specified amounts, as well as any product which results, directly or indirectly, from combination of the specified ingredients in the specified amounts.

The combination of the present invention is defined as follows:

Compound A and a compound which acts by reducing food intake when administered to a subject, or pharmaceutically acceptable salts thereof. Preferably, the combination comprises Compound A and Ob protein. Most preferably, the combination comprises Compound A and human Ob protein.

The β_3 agonist, Compound A, is synthesized as shown in Example 1, below, and as shown in Example 70 of U.S. Patent No. 5,561,142, issued October 1, 1996, hereby incorporated by reference.. Compound A is (R)-N-[4-[2-[[2-Hydroxy-2-(pyridin-3-yl)ethyl]amino]ethyl]-phenyl]-4-[4-(3-cyclopentylpropyl)-5-tetrazolon-1-yl]benzenesulfonamide, or a pharmaceutically acceptable salt thereof. Preferably, the dihydrochloride salt of Compound A is utilized in the combination.

Compounds which reduce food intake include the Ob protein (i.e., leptin). As used herein, the terms "Ob protein," "OB protein" and "ob protein" all refer to the same protein and are also synonymous with the protein referred to as "leptin." Preferably, the

combination of the present invention comprises Compound A and the Ob protein, or pharmaceutically acceptable salts thereof.

The Ob protein is obtained by expression of the recently discovered Ob gene. Expression of mouse Ob gene product in Drosophila Schneider 2 (S2) cells is described in Example 13, below. Based on the published sequence of the human Ob cDNA [Y. Zhang et al.. *Nature* **372**, 425-432 (1994); R.V. Considine et al., *J. Clin. Invest.* **95**, 2986-2988 (1995)], one of ordinary skill in the art could isolate human cDNA and obtain human Ob protein by expression of the human Ob cDNA in Drosophila S2 cells according to the protocol of Example 13. Similarly, the Ob protein may also be expressed in a bacterial expression system such as *E. coli* or a yeast system and purified by one of ordinary skill in the art.

In addition to the combination comprising Compound A and the Ob protein, compounds which cause increased expression of the Ob protein (for example, glucocorticoids, *see* P. De Vos, *J. Biol. Chem.* **270**(27), 15958-15961 (1995)) are also useful in combination with Compound A for treating obesity and diabetes. Further included within the invention are derivatives of Ob protein in which the protein is truncated to produce a small peptide and/or one or more amino acids are deleted, added, substituted or modified but which derivatives maintain the biological effect on feeding behavior and food intake. Examples of leptin derivatives (e.g., truncated forms of leptin) which are useful in the present invention include U.S. Patent Nos. 5,552,524; 5,552,523; 5,552,522; 5,521,283; and PCT International application publication nos. WO 96/23513; WO 96/23514; WO 96/23515; WO 96/23516; WO 96/23517; WO 96/23518; WO 96/23519; WO 96/23520, all published on August 8, 1996.

The pharmaceutically acceptable salts of the present invention (in the form of water- or oil-soluble or dispersible products) include the conventional non-toxic salts or the quaternary ammonium salts which are formed, e.g., from inorganic or organic acids or bases. Examples of such acid addition salts include acetate, adipate, alginate,

aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptanoate, glycerophosphate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, oxalate, pamoate, pectinate, persulfate, 3-phenylpropionate, picrate, pivalate, propionate, succinate, tartrate, thiocyanate, tosylate, and undecanoate. Base salts include ammonium salts, alkali metal salts such as sodium and potassium salts, alkaline earth metal salts such as calcium and magnesium salts, salts with organic bases such as dicyclohexylamine salts, N-methyl-D-glucamine, and salts with amino acids such as arginine, lysine, and so forth. Also, the basic nitrogen-containing groups may be quaternized with such agents as lower alkyl halides, such as methyl, ethyl, propyl, and butyl chloride, bromides and iodides; dialkyl sulfates like dimethyl, diethyl, dibutyl; and diamyl sulfates, long chain halides such as decyl, lauryl, myristyl and stearyl chlorides, bromides and iodides, aralkyl halides like benzyl and phenethyl bromides and others. Other pharmaceutically acceptable salts include the sulfate salt ethanolate and sulfate salts.

The pharmaceutically acceptable salts of the composition of the instant invention include the composition wherein one of the individual components of the combination is in the form of a pharmaceutically acceptable salt, or the composition wherein all of the individual components are in the form of pharmaceutically acceptable salts (wherein the salts for each of the components can be the same or different), or a pharmaceutically acceptable salt of the combined components (i.e., a salt of the composition). In one embodiment of the present invention, the hydrochloride salt of the composition is utilized.

The pharmaceutically acceptable esters in the present invention refer to non-toxic esters, preferably the alkyl esters such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl or pentyl esters, of which the methyl ester is preferred. However, other esters such as phenyl-C_{1-5} alkyl may be employed if desired.

Esterification of alcohols, such as Compound A of the present invention, is performed by a variety of conventional procedures, including reacting the alcohol group with the appropriate anhydride, carboxylic acid or acid chloride. These reactions, as well as other methods of esterification of alcohols, are readily apparent to the skilled artisan.

Reaction of the alcohol with the appropriate anhydride is carried out in the presence of an acylation catalyst, such as 4-DMAP (4-dimethylaminopyridine, also known as N,N-dimethylaminopyridine), pyridine, or 1,8-bis[dimethylamino]napthalene.

Reaction of the alcohol with the appropriate carboxylic acid is carried out in the presence of a dehydrating agent and, optionally, an acylation catalyst. The dehydrating agent, which serves to drive the reaction by the removal of water is selected from dicyclohexylcarbodiimide (DCC), 1-[3-dimethylaminopropyl]-3-ethylcarbodiimide (EDC) or other water soluble dehydrating agents.

Alternatively, reaction of the alcohol with appropriate carboxylic acid can also result in esterification, if performed instead in the presence of trifluoroacetic anhydride, and, optionally, pyridine. A further variant is reacting the alcohol with appropriate carboxylic acid in the presence of N,N-carbonyldiimidazole with pyridine.

Reaction of the alcohol with the acid chloride is carried out with an acylation catalyst, such as 4-DMAP or pyridine.

During any of the above methods for forming esters, it may be necessary and/or desirable to protect sensitive or reactive groups on any of the molecules concerned. This may be achieved by means of conventional protecting groups, such as those described in Protective Groups in Organic Chemistry, ed. J.F.W. McOmie, Plenum Press, 1973; and T.W. Greene & P.G.M. Wuts, Protective Groups in Organic Synthesis, John Wiley & Sons, 1991. The protecting groups may be removed at a convenient subsequent stage using methods known from the art.

In one aspect, the present invention provides a combination of compounds or a pharmaceutically acceptable ester thereof: or a

pharmaceutically acceptable salt thereof for use in the treatment of obesity in human or non-human animals.

The present invention further provides a combination of compounds, or a pharmaceutically acceptable ester thereof; or pharmaceutically acceptable salt thereof, for use in the treatment of hyperglycemia (diabetes) in human or non-human animals.

The disease diabetes mellitus is characterized by metabolic defects in production and utilization of glucose which result in the failure to maintain appropriate blood sugar levels. The result of these defects is elevated blood glucose or hyperglycemia. Research on the treatment of diabetes has centered on attempts to normalize fasting and postprandial blood glucose levels. Treatments have included parenteral administration of exogenous insulin, oral administration of drugs and dietary therapies.

Two major forms of diabetes mellitus are now recognized. Type I diabetes, or insulin-dependent diabetes, is the result of an absolute deficiency of insulin, the hormone which regulates glucose utilization. Type II diabetes, or insulin-independent diabetes (i.e., non-insulin-dependent diabetes mellitus), often occurs in the face of normal, or even elevated levels of insulin and appears to be the result of the inability of tissues to respond appropriately to insulin. Most of the Type II diabetics are also obese. The combination of the present invention is useful for treating both Type I and Type II diabetes. The combination is especially effective for treating Type II diabetes.

The combination of compounds of the present invention is useful in the treatment of obesity and diabetes. For these purposes, the combinations of the present invention may be administered orally, parenterally (including subcutaneous injections, intravenous, intramuscular, intrasternal injection or infusion techniques), by inhalation spray, or rectally, in dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants and vehicles.

Thus, in accordance with the combination of the present invention there is further provided a method of treating and a

pharmaceutical composition for treating obesity and diabetes. The treatment involves administering to a patient in need of such treatment a pharmaceutical composition comprising a pharmaceutical carrier and a therapeutically effective amount of each compound in the combination of the present invention.

These pharmaceutical compositions may be in the form of orally-administrable suspensions or tablets; nasal sprays; sterile injectable preparations, for example, as sterile injectable aqueous or oleaginous suspensions or suppositories.

In accordance with the methods of the present invention, the individual components of the combination can be administered separately at different times during the course of therapy or concurrently in divided or single combination forms. For example, in a two-component combination which is the β3 agonist, Compound A, and the Ob protein, treatment with Ob protein can commence prior to, subsequent to or concurrent with commencement of treatment with Compound A. Furthermore, the term administering also encompasses the use of prodrugs of the β3 agonist and/or Ob protein which convert in vivo to a selective β3 agonist or Ob protein or derivative thereof. The instant invention is therefore to be understood as embracing all such regimes of simultaneous or alternating treatment and the term "administering" is to be interpreted accordingly.

When any of the active ingredients (e.g. Compound A) are administered orally as a suspension, these compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and may contain microcrystalline cellulose for imparting bulk, alginic acid or sodium alginate as a suspending agent, methylcellulose as a viscosity enhancer, and sweeteners/flavoring agents known in the art. As immediate release tablets, these compositions may contain microcrystalline cellulose, dicalcium phosphate, starch, magnesium stearate and lactose and/or other excipients, binders, extenders, disintegrants, diluents and lubricants known in the art.

When administered by nasal aerosol or inhalation, these compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and may be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other solubilizing or dispersing agents known in the art.

The compounds utilized in the combination may also be administered in intravenous (both bolus and infusion), intraperitoneal, subcutaneous, topical with or without occlusion, or intramuscular form, all using forms well known to those of ordinary skill in the pharmaceutical arts. When administered by injection, the injectable solutions or suspensions may be formulated according to known art, using suitable non-toxic, parenterally-acceptable diluents or solvents, such as mannitol, 1,3-butanediol, water, Ringer's solution or isotonic sodium chloride solution, or suitable dispersing or wetting and suspending agents, such as sterile, bland, fixed oils, including synthetic mono- or diglycerides, and fatty acids, including oleic acid.

When rectally administered in the form of suppositories, these compositions may be prepared by mixing the drug with a suitable non-irritating excipient, such as cocoa butter, synthetic glyceride esters or polyethylene glycols, which are solid at ordinary temperatures, but liquidify and/or dissolve in the rectal cavity to release the drug.

The active ingredients of the combination (e.g., Compound A) of the present invention may be administered as a pharmaceutical composition, for example, with an inert diluent, or with an assimilable edible carrier, or they may be enclosed in hard or soft shell capsules, or they may be compressed into tablets, or they may be incorporated directly with the food of the diet. For oral therapeutic administration, which includes sublingual administration, these active compounds may be incorporated with excipients and used in the form of tablets, pills, capsules, ampules, sachets, elixirs, suspensions, syrups, and the like. Such compositions and preparations should contain at least 0.1 percent

of the active ingredients. The percentage of active ingredients in these compositions may, of course, be varied and may conveniently be between about 2 percent to about 60 percent of the weight of the unit. The amount of active ingredients in such therapeutically useful compositions is such that an effective dosage will be obtained. The active compounds can also be administered intranasally as, for example, liquid drops or spray.

The effective dosage of each of the active ingredients employed in the combination may vary depending on the particular compound employed, the mode of administration, the condition being treated and the severity of the condition being treated. Thus, the dosage regimen utilizing the compounds of the present invention is selected in accordance with a variety of factors including type, species, age, weight, sex and medical condition of the patient; the severity of the condition to be treated; the route of administration; the renal and hepatic function of the patient; and the particular compound thereof employed. A physician or veterinarian of ordinary skill can readily determine and prescribe the effective amount of the drug required to prevent, counter or arrest the progress of the condition. Optimal precision in achieving concentration of drug within the range that yields efficacy without toxicity requires a regimen based on the kinetics of the drug's availability to target sites. This involves a consideration of the distribution, equilibrium, and elimination of a drug.

The compounds of this invention can be administered to humans in the dosage ranges specific for each compound. When treating diabetes mellitus and/or hyperglycemia generally satisfactory results are obtained when Compound A, or a pharmaceutically acceptable salt thereof, is administered at a daily dosage of from about 0.001 milligram to about 100 milligram per kilogram of animal body weight, preferably given in a single dose or in divided doses two to six times a day, or in sustained release form. In the case of an adult human, the total daily dose will generally be from about 0.07 milligrams to about 350 milligrams. This dosage regimen may be adjusted to provide the optimal therapeutic response. The Ob protein is administered at a

daily dosage of from about 0.05 mg/kg to about 20 mg/kg, preferably injected in a single dose or in divided doses 2 to 3 times per day, or in sustained release form. Preferably, the daily dosage of Ob protein is from about 0.05 mg/kg to about 5 mg/kg. It will be understood, however, that the specific dose level and frequency of dosage for any particular patient may be varied and will depend upon a variety of factors including the activity of the specific compound employed, the metabolic stability and length of action of that compound, the age, body weight, general health, sex, diet, mode and time of administration, rate of excretion, drug combination, the severity of the particular condition, and the host undergoing therapy.

When treating obesity, in conjunction with diabetes and/or hyperglycemia, or alone, generally satisfactory results are obtained when Compound A is administered at a daily dosage of from 0.01 milligram to about 100 milligrams per kilogram of animal body weight, preferably given in a single dose or in divided doses two to six times a day, or in sustained release form. In the case of an adult human, the total daily dose will generally be from about 0.7 milligrams to about 3500 milligrams. The Ob protein is administered at a daily dosage of from about 0.05 mg/kg to about 20 mg/kg, preferably given in a single dose or in divided doses 2 to 3 times per day, or as a constant infusion. Preferably, the daily dosage of Ob protein is from about 0.05 mg/kg to about 5 mg/kg. This dosage regimen may be adjusted to provide the optimal therapeutic response. It will be understood, however, that the specific dose level and frequency of dosage for any particular patient may be varied and will depend upon a variety of factors including the activity of the specific compound employed, the metabolic stability and length of action of that compound, the age, body weight, general health, sex, diet, mode and time of administration, rate of excretion, drug combination, the severity of the particular condition, and the host undergoing therapy.

The tablets, pills, capsules, and the like may also contain a binder such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch,

potato starch, alginic acid; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, lactose or saccharin. When a dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier such as a fatty oil.

Various other materials may be present as coatings or to modify the physical form of the dosage unit. For instance, tablets may be coated with shellac, sugar or both. A syrup or elixir may contain, in addition to the active ingredient, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and a flavoring such as cherry or orange flavor.

These active compounds may also be administered parenterally. Solutions or suspensions of these active compounds can be prepared in water suitably mixed with a surfactant such as hydroxy-propylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols and mixtures thereof in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (e.g. glycerol, propylene glycol and liquid polyethylene glycol), suitable mixtures thereof, and vegetable oils.

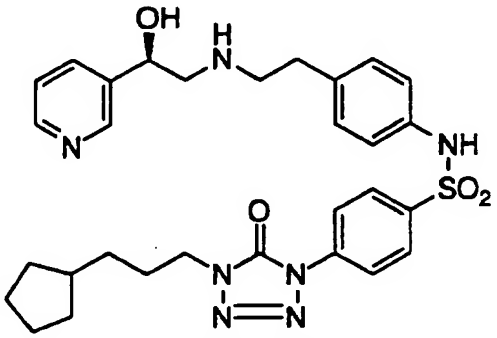
Abbreviations used in the instant specification, particularly the Schemes and Examples, are as follows:

Boc or BOC	=	t-butyloxycarbonyl
DBBA	=	dibromobarbituric acid
(-)-DIP-Cl	=	(-)-B-chlorodiisopinocampheylborane
DMF	=	N,N-dimethylformamide

DTT	=	dithiothreitol
EDTA	=	ethylenediaminetetraacetic acid
HPLC	=	high performance liquid chromatography
NCS	=	N-chlorosuccinimide
NMR	=	nuclear magnetic resonance
THF	=	tetrahydrofuran
TLC	=	thin layer chromatography
Tris	=	Tris(hydroxymethyl)aminomethane

The following examples are provided so that the invention might be more fully understood. They should not be construed as limiting the invention in any way.

EXAMPLE 1



Compound A

(R)-N-[4-[2-[[2-Hydroxy-2-(pyridin-3-yl)ethyl]amino]ethyl]phenyl]-4-[4-(3-cyclopentylpropyl)-5-tetrazolon-1-yl]benzenesulfonamide

A. Preparation of 3-cyclopentyl-1-iodopropane

To a solution of 5 g of commercially available 3-cyclopentyl-1-propanol in 20 mL of dichloromethane at 0°C was added 5 mL of dry triethylamine and 5 g of methanesulfonyl chloride. The mixture containing a heavy white precipitate of triethylamine hydrochloride was stirred an additional 5 min before 70 mL of diethyl

ether was added. The mixture was filtered through a glass fritted funnel to remove the precipitate and the filtrate was concentrated in vacuo to afford the mesylate. To this was added 10 g of sodium iodide and 30 mL of acetone. After 18 h at room temperature, the dark slurry was dissolved in water and extracted with dichloromethane. The extracts were combined and washed with excess aqueous sodium sulfite and dried over magnesium sulfate. Filtration and concentration of the dichloromethane solution gave the title compound:
^{1}H NMR ($CDCl_3$) δ 3.19 (t, 2H, J=7.1 Hz), 1.85 (m, 3H), 1.77 (m, 2H), 1.61 (m, 2H), 1.52 (m, 2H), 1.41 (m, 2H), 1.09 (m, 2H).

B. Preparation of 4-(3-cyclopentylpropyl)-1-phenyl-5-tetrazolon

To a solution of 600 mg of 1-phenyl-5-tetrazolone (for the synthesis of this compound see: Horwitz, J. P.; Fisher, B. E.; Tomasewski, A. J. *J. Amer. Chem. Soc.* **81**, 3076 (1959)) in 2 mL of N,N-dimethylformamide (DMF) was added 280 mg of powdered 85% potassium hydroxide followed by 870 mg of 3-cyclopentyl-1-iodopropane from Step A. The mixture was stirred at 80°C for 18 h and then quenched by addition of water. The product was extracted with dichloromethane and purification by flash chromatography on silica gel (9:1 hexane:ethyl acetate) to afford the title compound: ^{1}H NMR ($CDCl_3$) δ 7.96 (d, 2H, J=8.5 Hz), 7.50 (t, 2H, J= 7.5 Hz), 7.37 (t, 1H, J=7.5 Hz), 4.02 (t, 2H, J=7.1 Hz), 1.90 (m, 3H), 1.79 (m, 2H), 1.61 (m, 2H), 1.53 (m, 2H), 1.41 (m, 2H), 1.10 (m, 2H).

C. Preparation of 4-(3-cyclopentylpropyl)-1-(4-nitrophenyl)-5-tetrazolone

To a solution of 7 g of 4-(3-cyclopentylpropyl)-1-phenyl-5-tetrazolone from Step B in 50 mL of dry acetonitrile was added, in one portion, 4.2 g of 95% nitronium tetrafluoroborate with rapid stirring. The reaction mixture was stirred at room temperature for 30 min before an additional 2 g of nitronium tetrafluoroborate was added to react with detectable starting material. The mixture was stirred an

additional 30 min before addition of water and extraction with ethyl acetate. The combined ethyl acetate extracts were washed with aqueous sodium bicarbonate and dried over magnesium sulfate. Filtration and concentration of the filtrate gave an orange oil. Flash chromatographic separation on silica gel using 4:1 hexane:ethyl acetate afforded the para-nitrophenyl title compound as a solid (Rf=0.7) and a minor amount of the ortho-nitrophenyl product as an oil (Rf=0.2): Para-nitro product ^{1}H NMR ($CDCl_3$) δ 8.35 (ABq, 4H, Jab= 9.3 Hz), 4.06 (t, 2H, J=7.1 Hz), 1.93 (m, 3H), 1.80 (m, 2H), 1.63 (m, 2H), 1.54 (m, 2H), 1.43 (m, 2H), 1.10 (m, 2H); Ortho-nitro product ^{1}H NMR ($CDCl_3$) δ 8.13 (dd, 1H, J=1.1, 8.3 Hz), 7.81 (dt, 1H, J= 1.3, 7.8 Hz), 7.72 (dd, 1H, J=1.1, 8.0 Hz), 7.67 (dt, 1H, J=1.4, 7.6 Hz), 4.03 (t, 2H, J= 7.1 Hz), 1.92 (m, 3H), 1.80 (m, 2H), 1.61 (m, 2H), 1.53 (m, 2H), 1.42 (m, 2H), 1.10 (m, 2H).

D. Preparation of 1-(4-aminophenyl)-4-(3-cyclopentyl-propyl)-5-tetrazolone

To a solution of 6.23 g of 4-(3-cyclopentylpropyl)-1-(4-nitrophenyl)-5-tetrazolone from Step C in 250 mL of ethanol was added 1.8 g of 10% palladium on carbon. The mixture was stirred under a 1 atmosphere pressure of hydrogen provided by a balloon for 3-6 h. The catalyst was then removed by filtration through a plug of silica gel. The filtrate was concentrated in vacuo to yield the title compound as a waxy solid: ^{1}H NMR ($CDCl_3$) δ 7.64 (d, 2H, J=8.9 Hz), 6.77 (d, 2H, J= 8.9 Hz), 4.01 (t, 2H, J= 7.3 Hz), 3.83 (br s, 2H), 1.90 (m, 3H), 1.79 (m, 2H), 1.60 (m, 2H), 1.54 (m, 2H), 1.41 (m, 2H), 1.10 (m, 2H).

E. Preparation of 4-[4-(3-cyclopentylpropyl)-5-tetrazolon-1-yll-benzenesulfonyl chloride

To a solution of 32 mL of concentrated hydrochloric acid and 8 mL of glacial acetic acid at -20°C was added 5.42 g of powdered 1-(4-aminophenyl)-4-(3-cyclopentylpropyl)-5-tetrazolone from Step D. The mixture was stirred for 5 min before a solution of 1.6 g of sodium nitrite in 10 mL of water was added at a rate which kept the temperature from rising above -10°C. The diazonium salt mixture was

stirred an additional 40 min at -20°C and then added in one portion to a solution of 32 mL of glacial acetic acid containing 690 mg of copper (I) chloride saturated with sulfur dioxide at 0°C. The resultant reaction mixture instantly changed in color from a dark green with nitrogen evolution to a lime green slurry as time progressed. The reaction mixture was allowed to warm from 0°C to room temperature over 50 min. The mixture was then poured into ice-water and extracted with ethyl acetate. The combined organic extracts were washed once with cold water, dried over magnesium sulfate, filtered, and concentrated in vacuo to remove acetic acid. The crude sulfonyl chloride was purified by flash chromatography (silica gel, dichloromethane) to yield the title compound as a white solid: ^{1}H NMR ($CDCl_3$) δ 8.37 (d, 2H, J= 9.1 Hz), 8.20 (d, 2H, J=8.6 Hz), 4.06 (t, 2H, J= 7.1 Hz), 1.93 (m, 3H), 1.81 (m, 2H), 1.62 (m, 2H), 1.55 (m, 2H), 1.41 (m, 2H), 1.11 (m, 2H).

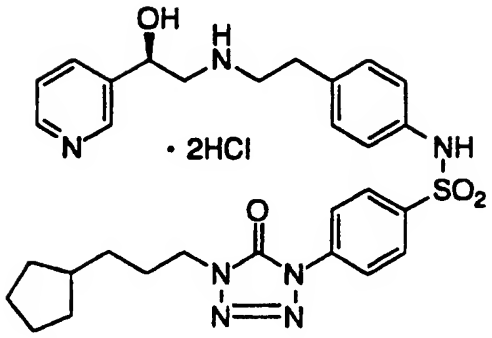
F. Preparation of (R)-N-[4-[2-[N-(1,1-dimethylethoxy-carbonyl)-N-[2-hydroxy-2-(pyridin-3-yl)ethyl]amino]ethyl]phenyl]-4-[4-(3-cyclopentyl-propyl)-5-tetrazolon-1-yl]benzenesulfonamide

To 2.84 g of the product from Example 7 was added 30 mL of dichloromethane and 3.01 g of 4-[4-(3-cyclopentylpropyl)-5-tetrazolon-1-yl]benzenesulfonyl chloride from Step E followed by 5 mL of dry pyridine. The orange solution was stirred at room temperature for 12 h and TLC showed no starting material left. The mixture was concentrated to dryness and the residual solid foam was taken up in dichloromethane and purified by flash chromatography on silica gel (4:6 acetone:hexane) 3 times to obtain the title compound: ^{1}H NMR (CD_3OD) δ 8.46 (d, 1H, J= 9.2 Hz), 8.41 (m, 1H, J= 4.8 Hz), 8.07 (d, 2H, J= 6.9 Hz), 7.86 (d, 2H, J=8.3 Hz), 7.80 (dd, 1H, J= 6.9, 24.3 Hz), 7.40 (m, 1H, J= 5.5 Hz), 7.02 (m, 4H), 4.85 (m, 1H), 4.01 (t, 2H, J= 7.1 Hz), 3.43-3.10 (m, 4H), 2.72 (m, 2H), 1.87 (m, 3H), 1.78 (m, 2H), 1.62 (m, 2H), 1.53 (m, 2H), 1.30 (d, 9H), 1.10 (m, 2H).

G. Preparation of (R)-N-[4-[2-[[2-hydroxy-2-(pyridin-3-yl)ethyl]amino]-ethyl]phenyl]-4-[4-(3-cyclopentylpropyl)-5-tetrazolon-1-yl]benzene-sulfonamide

A 3.1-g sample of Boc derivative from Step F was dissolved in 50 mL of methanol and 10 mL of concentrated hydrochloric acid. The solution was heated at 50°C for 1 h. A precipitate of the hydrochloride salt was obtained. This was cooled and basified with excess sodium bicarbonate solution and extracted with ethyl acetate. The combined extracts were dried over sodium sulfate, filtered, and concentrated in vacuo. The residual solid was purified by flash chromatography (silica gel, 9:1 dichloromethane:methanol) to yield the title compound as a glass:
^{1}H NMR (CD_3OD) δ 8.51 (d, 1H, J= 2.1 Hz), 8.42 (dd, 1H, J= 1.6, 4.8 Hz), 8.09 (d, 2H, J= 8.9 Hz), 7.86 (d, 2H, J= 8.7 Hz), 7.81 (d, 1H, J= 7.7 Hz), 7.39 (dd, 1H, J= 4.6, 7.8 Hz), 7.05 (ABq, 4H, Jab= 8.5 Hz), 4.79 (m, 1H), 3.97 (t, 2H, J= 7.1 Hz), 2.90-2.70 (m, 6H), 1.90-1.74 (m, 5H), 1.61 (m, 2H), 1.53 (m, 2H), 1.37 (m, 2H), 1.07 (m, 2H).

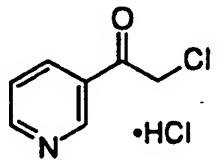
EXAMPLE 2



(R)-N-[4-[2-[[2-Hydroxy-2-(pyridin-3-yl)ethyl]amino]ethyl]phenyl]-4-[4-(3-cyclopentylpropyl)-5-tetrazolon-1-yl]benzenesulfonamide dihydrochoride

To 1.56 g of (R)-N-[4-[2-[[2-hydroxy-2-(pyridin-3-yl)ethyl]amino]ethyl]phenyl]-4-[4-(3-cyclopentylpropyl)-5-tetrazolon-1-yl]benzenesulfonamide, the free base from Example 1, was added 75 mL of methanol and 3 mL of 2 N hydrochloric acid. The solution was concentrated to dryness in vacuo and the residual salt was redissolved in 30 mL of methanol and 100 mL of boiling ethanol. Upon cooling (ice bath), crystallization occurred to afford the title compound as a white crystalline solid: mp 198-202°C (decomp.); ^{1}H NMR (CD_3OD) δ 8.99 (br s, 1H), 8.86 (d, 1H, J = 5.5 Hz), 8.74 (d, 1H, J = 8.0Hz), 8.14 (dd, 1H, J = 5.5, 8.0 Hz), 8.08 (d, 2H, J = 9 Hz), 7.89 (d, 2H, J = 9 Hz), 7.15 (ABq, 4H, Jab= 8.4 Hz), 5.35 (dd, 1H, J = 2.5, 9.5 Hz), 4.00 (t, 2H, J = 7.1 Hz), 3.47 (dd, 1H, J = 3.0, 12.8 Hz), 3.35-3.22 (m, 3H), 2.99 (m, 2H), 1.87 (m, 3H), 1.79 (m, 2H), 1.61 (m, 2H), 1.53 (m, 2H), 1.39 (m, 2H), 1.10 (m, 2H).

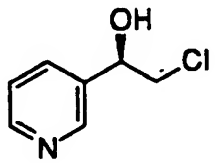
EXAMPLE 3



3-(2-Chloroacetyl)pyridine hydrochloride

To a solution of 12 g (11 mL, 100 mmol) of 3-acetylpyridine in 100 mL of ethyl ether was added 100 mL of 1 M ethereal hydrogen chloride. The resultant precipitate was filtered and 15.0 g (95.2 mmol) was collected and placed in a 500-mL round bottom flask equipped with a magnetic stir bar. To this was added 95 mL of 1 M hydrogen chloride in acetic acid. After the mixture was stirred until all the solid had dissolved, 12.7 g (95.2 mmol) of N-chlorosuccinimide (NCS) was added in one portion. The solution turned yellow and the NCS gradually dissolved. After 4 h, a white precipitate had formed. The mixture was allowed to stir for 2.5 days. It was then filtered. The solid collected was washed with 10 mL of acetic acid and 200 mL of ethyl ether to give the title compound as a white solid: ^{1}H NMR (200 MHz, d$_6$-DMSO) δ 9.22 (t, 1H, J = 1 Hz), 8.29 (dd, 1H, J = 1.6, 5.1 Hz), 8.55 (td, 1H, J = 2, 8.1 Hz), 7.82 (ddd, 1H, J = 0.8, 5.1, 8.1 Hz), 5.27 (s, 2H).

EXAMPLE 4

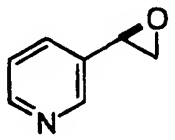


(R)–α–Chloromethyl-3-pyridinemethanol

To a stirred solution of 3.67 g (11.5 mmol) of (-)-B-chlorodiisopinocampheylborane [(-)-DIP-Cl] in 11 mL of THF at

-25 °C was added a slurry of 1.00 g (5.21 mmol) of the product from Example 3 in 5 mL of THF via a cannula. Following the addition of 0.80 mL (5.79 mmol) of triethylamine, the reaction mixture was stirred at -25 °C for 4 days. To the mixture was added 10 mL of water which was then allowed to warm to room temperature. To the mixture was added 20 mL of ethyl acetate and the organic phase separated. The aqueous phase was neutralized with saturated $NaHCO_3$ solution then extracted six times with ethyl acetate. The combined organic phase was concentrated in vacuo to afford a yellow oil. Flash chromatography (silica gel, 75 - 100% ethyl acetate-hexanes) afforded of the title compound as a pale yellow oil: ^1H NMR (400 MHz, CD_3OD) δ 8.58 (d, 1H, J = 1.8 Hz), 8.46 (dd, 1H, J = 4.9, 1.5 Hz), 7.90 (d, 1H, J = 7.9 Hz), 7.44 (dd, 1H, J = 7.9, 4.9 Hz), 4.93 (m, 1H), 3.75 (m, 2H).

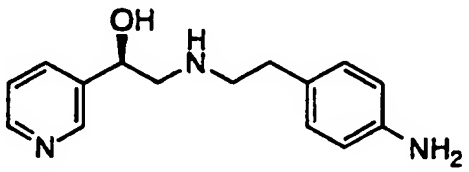
EXAMPLE 5



(R)-(Pyrid-3-yl)oxirane

To a solution of 557 mg (3.55 mmol) of the product from Example 4 in 16 mL of acetone was added 1.80 g of potassium carbononate. The mixture was heated at reflux for 20 h, then cooled to room temperature. The mixture was filtered and the filtrate evaporated in vacuo. Flash chromatography (silica gel, 2% methanol-methylene chloride) afforded the title compound as a pale yellow oil: ^1H NMR (200 MHz, $CDCl_3$) δ 8.54 (m, 2H), 7.52 (m, 1H), 7.24 (m, 1H), 3.86 (dd, 1H, J = 4.0, 2.5 Hz), 3.17 (dd, 1H, J = 5.4, 4.0 Hz), 2.80 (dd, 1H, J = 5.4, 2.5 Hz).

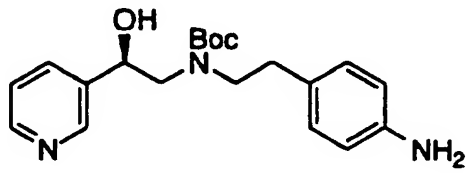
EXAMPLE 6



(R)-N-[2-[4-(Aminophenyl)]ethyl]-2-hydroxy-2-(pyrid-3-yl)ethylamine

To a stirred solution of 377 mg (2.44 mmol) of 4-aminophenethylamine in 10 mL of methanol was added a solution of 300 mg (2.48 mmol) of the product from Example 5 in 15 mL of methanol. The mixture was heated at reflux for 16 h, then cooled to room temperature. The methanol was removed in vacuo and the residue chromatographed (silica gel, 6 - 8% methanol, 1% ammonia-methylene chloride) to afford the title compound together with a mixture that was rechromatographed (5% methanol, 1% ammonia-methylene chloride) to give additional title compound as an off-white solid: ^{1}H NMR (500 MHz, CD_3OD) δ 8.52 (d, 1H, J = 1.8 Hz), 8.43 (dd, 1H, J = 4.8, 1.4 Hz), 7.81 (m, 1H), 7.40 (m, 1H), 6.95 (d, 2H, J = 8.3 Hz), 6.67 (d, 2H, J = 8.3 Hz), 4.81 (m, 1H), 2.90-2.65 (m, 6H).

EXAMPLE 7



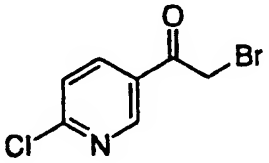
(R)-N-[2-[4-(aminophenyl)]ethyl]-2-hydroxy-2-(pyrid-3-yl)ethylcarbamic acid 1,1-dimethylethyl ester

A solution of 386 mg (1.77 mmol) of di-tert-butyl dicarbonate in 3.5 mL of THF was added, via a cannula, to a stirred slurry of 456 mg (1.77 mmol) of the product from Example 6 in 3.6 mL of THF cooled to 0°C. The yellow solution was stirred at 0°C for 3

h, then the THF was removed in vacuo. Flash chromatography (silica gel, 10% methanol, 1% ammonia-methylene chloride) afforded the title compound as an off white solid: ^{1}H NMR (500 MHz, CD_3OD, mixture of rotomers) δ 8.45 (m, 2H), 7.83 (d, 0.6H, J = 7.4 Hz), 7.78 (d, 0.4H, J = 6.9 Hz), 7.41 (m, 1H), 6.94 (d, 0.8H, J = 8.0 Hz), 6.89 (d, 1.2H, J = 7.8 Hz), 6.66 (d, 2H, J = 7.3 Hz), 4.89 (m, 1H), 3.42-3.21 (m, 4H), 2.67 (m, 2H), 1.39 (s, 5.4H), 1.36 (s, 3.6H).

An alternative synthesis of the aniline derivative in Example 7 is illustrated in Examples 8-12:

EXAMPLE 8

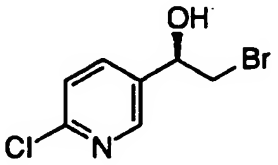


2-Chloro-5-(2-bromoacetyl)pyridine

A solution of 784 mg of 2-chloro-5-acetylpyridine in 10 mL of THF was added via canula to a solution of 1.44 g of dibromobarbituric acid (DBBA) in 10 mL of THF. The resultant solution was heated at 50-55°C for 12 h, and then an additional 0.72 g DBBA was added. After stirring at 50-55°C for 2.5 more hours, 0.36 g DBBA was added. The mixture was allowed to stir for 2 h, at which point NMR analysis of an aliquot indicated 87% conversion. The reaction mixture was cooled, diluted with ethyl acetate, washed with two portions of saturated aqueous sodium bicarbonate, water, and brine, dried over magnesium sulfate and concentrated. Purification by flash chromatography (silica gel, 15% ethyl acetate/hexane) provided the title compound as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.96 (d, 1H, J = 2.6 Hz), 8.21 (dd, 1H, J = 2.5, 8.3 Hz), 7.46 (d, 1H, J = 8.4 Hz), 4.37 (s, 2H). The NMR also indicated the presence of the

corresponding 2-bromo derivative. The ~4:1 mixture was carried on through the synthesis.

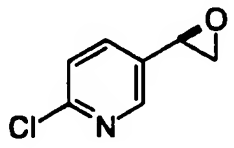
EXAMPLE 9



(R)-α-Bromomethyl-3-(6-chloropyridine)methanol

To a solution of 602 mg (1.88 mmol) of (-)-DIP-Cl in 0.5 mL of THF at -25°C was added via canula 200 mg of ketone from Example 8 in 1.5 mL of THF at -25°C. The reaction mixture was allowed to stir at -25°C for 17 h. It was then quenched by the addition of water and extracted with ether. The ether phase was diluted with ethyl acetate, washed with two portions of saturated aqueous sodium bicarbonate, water, and brine, dried over magnesium sulfate and concentrated. Purification by flash chromatography (silica gel, 15 and 25% ethyl acetate/hexane) gave the title compound: ^{1}H NMR (400 MHz, CDCl3) δ 8.38 (d, 1H), 7.70 (dd, 1H), 7.32 (d, 1H), 4.97 (m, 1H), 3.61 (dd, 1H), 3.50 (dd, 1H), 2.85 (d, 1H).

EXAMPLE 10

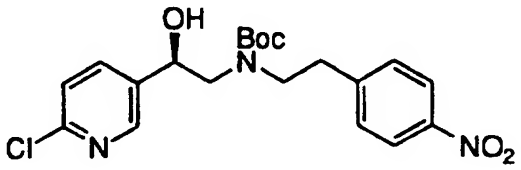


(R)-(2-chloropyrid-5-yl)oxirane

To a solution of 100 mg of bromoalcohol from Example 9 in 2 mL of 1:1 THF:water was added 1 mL of 5 N aqueous sodium hydroxide solution. The mixture was allowed to stir for 10 min. It was then extracted with three portions of dichloromethane. The combined

organic phases were washed with two portions of water and brine, dried over magnesium sulfate, and concentrated to give the title compound which was used without further purification: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 (d, 1H), 7.48 (dd, 1H), 7.29 (d, 1H), 3.86 (dd, 1H), 3.18 (dd, 1H), 2.78 (dd, 1H).

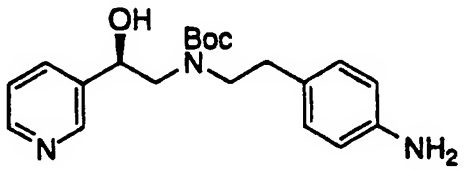
EXAMPLE 11



(R)-N-[2-[4-(Nitrophenyl)]ethyl]-2-hydroxy-2-(2-chloropyrid-5-yl)ethylcarbamic acid 1,1-dimethylethyl ester

Following the procedure outlined in Examples 6 and 7, the title compound was prepared from the epoxide from Example 10 and 4-nitrophenylethylamine: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (d, 1H, J = 1.3 Hz), 8.13 (d, 2H, J = 8.6 Hz), 7.66 (br m, 1H), 7.30 (d, 2H, J = 8.1 Hz), 7.27 (br m, 1H), 4.94 (br m), 3.38 (br m, 4H), 2.84 (br m, 2H), 1.40 (s, 9H).

EXAMPLE 12



(R)-N-[2-[4-(aminophenyl)]ethyl]-2-hydroxy-2-(pyrid-3-yl)ethylcarbamic acid 1,1-dimethylethyl ester

To a solution of 80 mg (0.19 mmol) of the nitro compound from Example 11 in 2 mL of ethanol was added 0.114 mL (0.57 mmol) of 5 N aqueous sodium hydroxide solution and 20 mg of raney nickel.

The reaction mixture was shaken at room temperature under 45 psi hydrogen for 16 h. The mixture was neutralized with saturated aqueous sodium phosphate monobasic and extracted with three portions of ethyl acetate. The combined organic phases were washed with water and brine, dried (magnesium sulfate), and concentrated to give the title compound which was identical to the sample prepared in Example 7.

EXAMPLE 13

Expression of mouse Ob gene product in Drosophila S2 cells

Total white adipose RNA was isolated from Swiss-Webster mice and first strand cDNA synthesized. Using the polymerase chain reaction (PCR), the coding region of the ob cDNA was isolated as 2 overlapping fragments using the following primer sets (5' CAGTGAGCCCCAAGAAGAGG 3' (SEQ. I.D. NO: 1), 5' TCCAGGTCATTGGCTATCTG 3' (SEQ. I.D. NO: 2)) and (5' ATTCCTGGGCTTCAGGGGATTCTGAGTTTC 3' (SEQ. I.D. NO: 3), 5' GCGTGTACCCACGGAGGAAC 3' (SEQ. I.D. NO: 4)). The resulting 380 bp and 626 bp fragments were purified and used as templates in a subsequent PCR reaction with primers 5' AAGAATTCATGTTGCTGGAGACCCCTGTGTC 3' (SEQ. I.D. NO: 5) and 5' AAGGATCCTCAGCATTCAGGGCTAACATC 3' (SEQ. I.D. NO: 6).

The final 501 bp fragment was sequenced using dye-primer chemistry on a Perkin-Elmer/Applied Biosystems 373A sequencer. The deduced amino acid sequence encoded by this cDNA was identical to that previously described. [Y. Zhang et al., *Nature* **373**, 425-432 (1994)]. The 501 bp fragment encoding the ob gene product was subcloned via EcoR1 and BamH1 sites into plasmid pRmHa3 [T.A. Bunch et al., *Nucleic Acids Research* **16**, 1043-1061 (1988)]. Drosophila S2 cells were cotransfected with pUChsneo [H. Steller and V. Pirrotta, *EMBO Journal* **4**, 167-171 (1985)] and the ob expression or control (pRmHa3) plasmid by $CaPO_4$ precipitation. A polyclonal population of transfected cells was selected with 1

mg/ml G418 and grown under serum free conditions in EXCELL 401 medium (JRH Bioscience). Cells were seeded at 2 x 10^6 cells/ml and $CuSO_4$ added to a final concentration of 1 mM. After 7 days, cells were removed by centrifugation and the supernatants filtered through a 0.45 μM filter.

Partial purification of the ob protein:

Proteins in the supernatant were concentrated by precipitation with 50 percent $(NH_4)SO_4$. The precipitate was dissolved in Buffer A (20mM Tris pH 8, 1mM DTT and 1mM EDTA) and desalted over PD-10 columns (Pharmacia) equilibrated in Buffer A. Proteins in the PD-10 eluate were subjected to Mono Q chromatography (Pharmacia) and eluted with a 0-200 mM gradient of NaCl in Buffer A. Peak fractions of ob immunoreactivity (centered at 100 mM NaCl) were identified using antiserum 103-2. Based on densitometric scanning of polyacrylamide gel containing pooled fractions eluted from the Mono Q column, the ob protein was 30 percent pure after this step. 0.5 to one milligram of this partially purified ob protein preparation was obtained per liter of S2 cells. Peak fractions were analyzed by HPLC electrospray mass spectrometry using a C_4 column (1 x 100 mm) eluted in a linear gradient of acetonitrile (zero to sixty-seven percent in 10mM trifluoroacetic acid. This reverse phase HPLC/mass spectrometry analysis demonstrated the immunoreactive protein to have a molecular mass of 16,004 Daltons, identidical to that predicted for the ob gene product after cleavage of the N terminal signal sequence between amino acids 21 and 22.

EXAMPLE 14

Immunological Methods

Antiserum 103-2 was isolated from a New Zealand white rabbit injected with a 4-branch multiple antigenic peptide corresponding to amino acids 22-41 of the mouse ob sequence, i.e.,

VPIQKVQDDTKTLIKTIVTR (SEQ. I.D. NO: 7). [Y. Zhang et al., *Nature* **372**, 425-432 (1994)]. Western blot analysis was performed on nitrocellulose (BA85m 0.45 μM pore size, Schleicher and Schuell Inc.). Immunodetection of the ob gene product was performed in TBS-T (20 mM Tris-Cl pH 7.6, 137 mM NaCl, 0.1% Tween 20) utilizing the ECL kit (Amersham). The secondary antibody (anti-rabbit Ig, horseradish peroxidase linked F(ab')2 fragment, Amersham) was used at 1:3000 dilution. A single immunoreactive protein with an apparent molecular weight of 14.5 kDa was identified.

EXAMPLE 15

In vivo study for combination therapy with Compound A and OB protein

Human patients are given injections (i.e., subcutaneous, intramuscular or intravenous) of 0.05 to 20 mg/kg total ob protein (human) in vehicle (ob protein treated), administered one to three times per day, together with or followed by 0.001 to 100 mg/kg Compound A dihydrochloride, administered orally or by injection one to three times per day. The first treatment is given on day 0, and the patients are treated daily for a six month period. A set of control patients are untreated (e.g., placebo) for comparison. Body weight data are collected each week. Statistical significance of body weight decreases is determined by performing a two-factor (group and day) analysis of variance (ANOVA) with repeated measures followed by a post hoc Least Squares Difference (LSD) test.

Determination of plasma insulin and glucose levels:

Blood is collected into heparinized capillary tubes on the day before the first treatment and daily for the first week. Thereafter, patients should individually monitor their own blood glucose daily (using commercially available kits). Blood is collected for laboratory analysis weekly at the same time that body weight measurements are

taken. Blood samples are analyzed (e.g., at a registered hospital or other GLP laboratory) for glucose and insulin levels.

While the foregoing specification teaches the principles of the present invention, with examples provided for the purpose of illustration, it will be understood that the practice of the invention encompasses all of the usual variations, adaptions, or modifications, as come within the scope of the following claims and its equivalents.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: SMITH, ROY G.

(ii) TITLE OF INVENTION: COMBINATION THERAPY FOR THE TREATMENT OF DIABETES AND OBESITY

(iii) NUMBER OF SEQUENCES: 7

(iv) CORRESPONDENCE ADDRESS:
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(E) COUNTRY: USA
(F) ZIP: 07065-0907

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: APPOLLINA, MARY A.
(B) REGISTRATION NUMBER: 34,087
(C) REFERENCE/DOCKET NUMBER: 19570Y

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (908) 594-3462
(B) TELEFAX: (908) 594-4720

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CAGTGAGCCC CAAGAAGAGG
20

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TCCAGGTCAT TGGCTATCTG
20

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATTCCTGGGC TTCAGGGGAT TCTGAGTTTC
30

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GCGTGTACCC ACGGAGGAAC
20

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

AAGAATTCAT GTTGCTGGAG ACCCCTGTGT C
31

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

AAGGATCCTC AGCATTCAGG GCTAACATC
29

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Val Pro Ile Gln Lys Val Gln Asp Asp Thr Lys Thr Leu Ile Lys Thr
1 5 10 15

Ile Val Thr Arg
20

WHAT IS CLAIMED IS:

1. A composition comprising a selective β3 agonist and a compound which modifies feeding behavior; and the pharmaceutically acceptable salts and esters thereof.

2. The composition of Claim 1, wherein the compound which modifies feeding behavior is Ob protein or a derivative thereof; and the pharmaceutically acceptable salts and esters thereof.

3. The composition of Claim 2, wherein the compound which modifies feeding behavior is human Ob protein or a derivative thereof; and the pharmaceutically acceptable salts and esters thereof.

4. The composition of Claim 3, wherein the β3 agonist is (R)-N-[4-[2-[[2-hydroxy-2-(pyridin-3-yl)ethyl]amino]ethyl]phenyl]-4-[4-(3-cyclopentylpropyl)-5-tetrazolon-1-yl]benzenesulfonamide; and the pharmaceutically acceptable salts and esters thereof.

5. The composition of Claim 4, wherein the β3 agonist is (R)-N-[4-[2-[[2-hydroxy-2-(pyridin-3-yl)ethyl]amino]ethyl]phenyl]-4-[4-(3-cyclopentylpropyl)-5-tetrazolon-1-yl]benzenesulfonamide dihydrochloride.

6. A method of treating obesity in a subject in need thereof which comprises administering to the subject a therapeutically effective amount of the composition of Claim 1.

7. A method of treating diabetes in a subject in need thereof which comprises administering to the subject a therapeutically effective amount of the composition of Claim 1.

8. A method of treating obesity in a subject in need thereof which comprises administering to the subject a therapeutically effective amount of the composition of Claim 4.

9. A method of treating diabetes in a subject in need thereof which comprises administering to the subject a therapeutically effective amount of the composition of Claim 4.

10. A pharmaceutical composition comprising a therapeutically effective amount of the composition of Claim 1 and a pharmaceutically acceptable carrier.

11. A pharmaceutical composition comprising a therapeutically effective amount of the composition of Claim 4 and a pharmaceutically acceptable carrier.

12. A composition comprising (R)-N-[4-[2-[[2-hydroxy-2-(pyridin-3-yl)ethyl]amino]ethyl]phenyl]-4-[4-(3-cyclopentylpropyl)-5-tetrazolon-1-yl]benzenesulfonamide and human Ob protein or a derivative thereof; and the pharmaceutically acceptable salts and esters thereof.

13. A method of treating obesity in a subject in need thereof which comprises administering to the subject a therapeutically effective amount of a selective β_3 agonist and a compound which modifies feeding behavior.

14. The method of treating obesity of Claim 13, wherein the selective β_3 agonist is Compound A, or a pharmaceutically acceptable salt thereof, and the compound which modifies feeding behavior is Ob protein, or a derivative thereof.

15. The method of treating obesity of Claim 14, wherein the selective β3 agonist is the dihydrochloride salt of Compound A and the Ob protein is human Ob protein or a derivative thereof.

16. A method of treating diabetes in a subject in need thereof which comprises administering to the subject a therapeutically effective amount of a selective β3 agonist and a compound which modifies feeding behavior.

17. The method of treating diabetes of Claim 16, wherein the selective β3 agonist is Compound A, or a pharmaceutically acceptable salt thereof, and the compound which modifies feeding behavior is Ob protein, or a derivative thereof.

18. The method of treating diabetes of Claim 17, wherein the selective β3 agonist is the dihydrochloride salt of Compound A and the Ob protein is human Ob protein or a derivative thereof.

19. A pharmaceutical composition made by combining a selective β3 agonist, a compound which modifies feeding behavior and a pharmaceutically acceptable carrier.

20. A process for making a pharmaceutical composition which comprises combining a selective β3 agonist, a compound which modifies feeding behavior and a pharmaceutically acceptable carrier.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/17444

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 31/44, 38/18, 38/17
US CL :514/12, 333
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/12, 333

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS
search terms: beta-3 agonist, Ob protein, leptin, obesity, diabetes

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y, P	US 5,561,142 A (FISHER et al) 01 October 1996, see column 48, lines 17-33 and the claims.	1-20
Y	HALAAS et al. Weight-Reducing Effects of the Plasma Protein Encoded by the Obese Gene. Science. 28 July 1995. Vol. 269. Pages 543-546, especially the abstract.	1-20

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 21 DECEMBER 1996	Date of mailing of the international search report 27 JAN 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Deborah Frese for WILLIAM JARVIS Telephone No. (703) 308-1235

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